

**PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

CHI LI LIU  
JEFFERSON C. LIEVENSE

Serial No.: 10/717,993

Filed: November 20, 2003

For: LACTIC ACID PRODUCING YEAST

Confirmation No.: 7643

Group Art Unit: 1652

Examiner: Mohammad Y. Meah

Attorney Docket: 2027.631000/RFE  
(2006310)

**CUSTOMER NO. 23720**

**APPEAL BRIEF**

Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Applicants hereby submit this Appeal Brief to the Board of Patent Appeals and Interferences in response to the final Office Action dated August 29, 2007.

The Director is authorized to deduct said fee under 37 C.F.R. §§ 1.16 to 1.21 from Williams, Morgan & Amerson, P.C. Deposit Account No. 50-0786/2027.631000RE.

I. REAL PARTY IN INTEREST

The real party in interest is Tate & Lyle Ingredients Americas, Inc., the successor to A. E. Staley Manufacturing Company, having a place of business at 2200 E. Eldorado Street, Decatur, Illinois, 62525.

II. RELATED APPEALS AND INTERFERENCES

None.

III. STATUS OF THE CLAIMS

Claims 24-128 have been canceled. The cancellation of claim 102 was made by Applicants in their paper submitted on October 23, 2007. Claims 1-23 and 129-130 are pending, rejected, and the subject of this appeal.

IV. STATUS OF AMENDMENTS

In response to the final Office Action dated August 29, 2007, Applicants amended claims 11, 13, and 16 to correct typographical errors. In the Advisory Action mailed November 13, 2007, the Examiner indicated these amendments would be entered for purposes of appeal.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Claim 1 is directed to a method of producing lactic acid (p. 11, lines 9-10). The method comprises a step of performing selection on a parent yeast strain that contains an exogenous lactate dehydrogenase gene (p. 11, lines 18-23). The exogenous lactate dehydrogenase gene encodes the amino acid sequence of a lactate dehydrogenase protein of an organism selected from the group consisting *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* (p. 24, lines 9-12). The

performing selection step yields an acid-tolerant (AT) yeast strain that is capable of growing in a minimal medium at a lower pH than the parent yeast strain (p. 4, lines 21-22; p. 26, lines 7-8).

The method also comprises a step of culturing in a minimal medium the acid-tolerant (AT) yeast strain (p. 4, lines 21-26), wherein the AT yeast strain produces less than about 1 ppm ethanol (p. 16, lines 17-19), wherein the exogenous lactate dehydrogenase gene is capable of being expressed in the AT yeast strain (p. 4, lines 21-26 and p. 11, lines 18-23), and wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity (p. 4, lines 21-26).

To summarize, the invention recited by claim 1 is a method comprising the steps of performing selection and culturing.

Claim 129 further limits claim 1 by reciting that the step of performing selection comprises a number of actions. The first action is growing the parent yeast strain aerobically in a minimal medium, to yield a parent yeast strain culture (p. 25, lines 14-15 and 19-20). The second action is approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid (p. 25, lines 21-23). The third action is removing an aliquot of the parent yeast strain culture when the parent yeast strain culture is at about the lowest pH (p. 25, lines 23-24). The fourth action is seeding a minimal medium with the aliquot of the parent yeast strain culture (p. 25, line 25). The fifth action is repeating the growing, approximating, removing, and seeding steps until a final lowest pH reaches a value lower than the lowest pH of the first approximating and first removing steps, to yield an acid-tolerant yeast strain (p. 25, lines 25-30).

To summarize, the invention recited by claim 129 is a method comprising the steps of performing selection (involving the actions of growing, approximating, removing, seeding, and repeating the previous four actions) and culturing.

Claim 130 is similar to claim 129, however, removing the aliquot is performed when the parent yeast strain culture ceases growth (p. 25, lines 23-24), and repeating does not repeat the growing action.

#### VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Are claims 1-9 and 12-23 anticipated under 35 U.S.C. §102(e) by Hause, *et al.*, US 2003/0228671 ("Hause")?

Are claims 1-10 and 12-23 anticipated under 35 U.S.C. §102(e) by Rajgarhia, *et al.*, US 2004/0029238 ("Rajgarhia")?

Are claims 129-130 unpatentable under 35 U.S.C. § 103(a) over Rajgarhia in view of Barnett, *et al.*, *Yeasts: characterization and identification*, 2<sup>nd</sup> ed., Cambridge University Press, ISBN 052135056, pp. 20-28 ("Barnett")?

#### VII. ARGUMENT

A. *Rejection of claims 1-9 and 12-23 as being anticipated under 35 U.S.C. §102(e) by Hause*

As is well known, for a reference to anticipate a claim, it must teach every element of the claim. MPEP 2131. The present claims, as pointed out above, recite a method comprising the steps of performing selection and culturing to yield an acid tolerant yeast strain. Hause does not teach every element of claim 1 or any claim dependent thereon.

Hause is directed to yeast containing a recombinant nucleic acid containing a lactate dehydrogenase (LDH) gene and a selection marker gene [0008, 0010], and to methods of integrating the recombinant nucleic acid into the yeast [0008]. Hause's integration method inserting the recombinant nucleic acid into the yeast in a manner whereby the LDH gene is integrated into the yeast's native genome adjacent to a targeted gene in the native genome, growing the yeast in a first medium wherein only yeast containing the selection marker gene will grow, growing the yeast in a second medium wherein yeast that spontaneously lose the selection marker gene can still grow, and obtaining yeast having the LDH gene and lacking both the selection marker gene and the targeted gene (*ibid.*). The selection marker genes contemplated by Hause include those that confer resistance to antibiotics, complement auxotrophic deficiencies of the cell, or supply critical nutrients not available from the first medium [0058]. Both the types of selection marker genes and their use, as contemplated by Hause, are well known to the person of ordinary skill in the art as a means of determining whether a target cell has been transformed with a recombinant nucleic acid molecule containing both the selection marker gene and a gene of interest, such as the LDH gene taught by Hause. In other words, Hause's selection marker gene allows the operator of Hause's method to determine which cells of *a yeast strain that did not contain an exogenous LDH gene prior to Hause's inserting step* acquired an exogenous LDH gene *during* Hause's inserting step.

The present claims, in contrast, *start with a yeast strain that already contains an exogenous LDH gene* and perform a method that yields a yeast strain that retains the exogenous LDH gene and is more acid tolerant than the starting yeast strain. In other words, the operator *begins* performance of the method of the present claims on a population of yeast that are the final product of Hause's method (or any other method of introducing a recombinant nucleic acid into a

yeast). Therefore, Hause does not teach a performing selection step as recited by present claim 1 and all claims dependent thereon.

Plainly, Hause does not teach every element of the present claims, and therefore, Applicants submit claims 1-9 and 12-23 are not anticipated under 35 U.S.C. §102(e) by Hause and the rejection should be withdrawn.

*B. Rejection of claims 1-10 and 12-23 as being anticipated under 35 U.S.C. §102(e) by Rajgarhia*

As is well known, for a reference to anticipate a claim, it must teach every element of the claim. MPEP 2131. The present claims, as pointed out above, recite a method comprising the steps of performing selection and culturing to yield an acid tolerant yeast strain. Rajgarhia does not teach every element of claim 1 or any claim dependent thereon.

Rajgarhia is directed to yeast containing a recombinant nucleic acid containing, for example, a lactate dehydrogenase (LDH) gene [0010], and to methods of inserting the recombinant nucleic acid into the yeast [0100-0101]. Rajgarhia does not discuss selection marker genes in detail, but refers to specific ones in the Examples, e.g., a gene for ampicillin resistance in *Escherichia coli* (Example 4, [0160]) and a gene for acrylate assimilation from *Clostridium propionicum* (Example 9, [0185]). Both the selection marker genes and their use, as contemplated by Rajgarhia, are well known to the person of ordinary skill in the art as a means of determining whether a target cell has been transformed with a recombinant nucleic acid molecule containing both the selection marker gene and a gene of interest, such as the LDH gene taught by Rajgarhia. In other words, Rajgarhia's selection marker gene allows the operator of Rajgarhia's method to determine which cells of *a yeast strain that did not contain an exogenous*

*LDH gene prior to Rajgarhia's inserting step* acquired an exogenous LDH gene *during* Rajgarhia's inserting step.

The present claims, in contrast, *start with a yeast strain that already contains an exogenous LDH gene* and perform a method that yields a yeast strain that retains the exogenous LDH gene and is more acid tolerant than the starting yeast strain. In other words, the operator *begins* performance of the method of the present claims on a population of yeast that are the final product of Rajgarhia's method (or any other method of introducing a recombinant nucleic acid into a yeast). Therefore, Rajgarhia does not teach a performing selection step as recited by present claim 1 and all claims dependent thereon.

Further, the Examiner has admitted that Rajgarhia does not teach a performing selection step, at p. 6 of the Detailed Action of August 29, 2007: "...Rajgarhia et al. do not teach a method of selection of AT yeast strain."

Plainly, Rajgarhia does not teach every element of the present claims, and therefore, Applicants submit claims 1-10 and 12-23 are not anticipated under 35 U.S.C. §102(e) by Rajgarhia and this rejection should be withdrawn.

*C. Rejection of claims 129-130 as being unpatentable under 35 U.S.C. § 103(a) over Rajgarhia in view of Barnett*

Rajgarhia has been discussed at length above. The cited pages from Barnett are from a chapter entitled "Laboratory methods for identifying yeasts." As is apparent from the introduction to the chapter (Barnett, p. 20), the chapter is directed to methods by which a yeast sample of unknown species can be identified as belonging to a known species. Upon reading Barnett, the person of ordinary skill in the art will know that a particular yeast species will grow

and/or exhibit certain morphological characteristics (*e.g.*, filamentous growth, ballistoconidia, and ascospores) under a first set of conditions (*e.g.*, medium composition, medium pH, and temperature) but not necessarily under a second set of conditions (differing in, *e.g.*, medium composition, pH, or temperature), and by examination of the unknown yeast under various conditions, the known species to which the unknown yeast belongs can be determined. The determination or identification performed by the person of ordinary skill in the art in light of Barnett is not a "selection" as the latter term is used by the present specification, despite the Examiner's allegation at p. 6 of the Detailed Action of August 29, 2007. As should be apparent, the person of ordinary skill in the art, following the teachings of Barnett, would be unable to generate a yeast of a desired known species from the unknown yeast presented to him, and therefore would not be "selecting" the yeast.

All that a combination of Rajgarhia and Barnett would provide to the person of ordinary skill in the art are techniques for identifying a yeast species either used by Rajgarhia, contemplated for use by Rajgarhia, or hypothetically contaminating a sample used by Rajgarhia. Knowing techniques for identifying a yeast species as taught by Barnett and applying them to the teachings of Rajgarhia does not provide the person of ordinary skill in the art with any guidance to undertake a performing selection step as recited by claim 1, let alone the listed actions of a performing selection step as recited by dependent claims 129-130.

Therefore, Applicants submit claims 129-130 are patentable under 35 U.S.C. § 103(a) over Rajgarhia in view of Barnett and this rejection should be withdrawn.

## VIII. CLAIMS APPENDIX

The claims that are the subject of the present appeal – claims 1-23 and 129-130 – are set forth in the attached "Claims Appendix."



IX. EVIDENCE APPENDIX

There is no separate Evidence Appendix for this appeal.

X. RELATED PROCEEDINGS APPENDIX

There is no Related Proceedings Appendix for this appeal.

XI. CONCLUSION

Applicants submit all pending claims 1-23 and 129-130 are in condition for allowance.

Respectfully submitted,

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January 23, 2008

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AGENT FOR APPLICANTS

## CLAIMS APPENDIX

Claim 1. (Previously presented) A method of producing lactic acid, comprising:  
performing selection on a parent yeast strain that contains an exogenous lactate dehydrogenase gene encoding the amino acid sequence of a lactate dehydrogenase protein of an organism selected from the group consisting *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* that is capable of being expressed in the parent yeast strain, to yield an acid-tolerant (AT) yeast strain that is capable of growing in a minimal medium at a lower pH than the parent yeast strain; and  
culturing in a minimal medium the acid-tolerant (AT) yeast strain, wherein the AT yeast strain produces less than about 1 ppm ethanol,  
wherein the exogenous lactate dehydrogenase gene is capable of being expressed in the AT yeast strain, and  
wherein a protein resulting from the expression of the exogenous lactate dehydrogenase gene has lactate dehydrogenase activity.

Claim 2. (Original) The method of claim 1, wherein the AT yeast strain is C<sub>2</sub> carbon source independent and is capable of producing lactic acid at a pH of less than about 3.5.

Claim 3. (Original) The method of claim 1, wherein the AT yeast strain is C<sub>2</sub> carbon source independent and is capable of producing lactic acid at a pH of less than about 2.8.

Claim 4. (Original) The method of claim 1, wherein the AT yeast strain is C<sub>2</sub> carbon source independent and is capable of producing lactic acid at a pH of less than about 2.3.

Claim 5. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing greater than about 50 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 6. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing between about 50 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 7. (Original) The method of claim 1, wherein the AT yeast strain is capable of producing between about 70 and 85 grams lactic acid/100 grams glucose when cultured in the minimal medium comprising glucose as a sole carbon source.

Claim 8. (Previously presented) The method of claim 1, wherein a culture broth resulting from the culturing of the AT yeast strain comprises less ppm of at least one of glycerol, erythritol, malic acid, pyruvic acid, succinic acid, formic acid, and fumaric acid than a culture broth resulting from the culturing of the parent strain in the same minimal medium under the same culture conditions.

Claim 9. (Original) The method of claim 1, wherein the AT yeast strain belongs to a genus selected from the group consisting of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, and *Kluyveromyces*.

Claim 10. (Original) The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae*.

Claim 11. (Previously presented) The method of claim 1, wherein the AT yeast strain is a *Saccharomyces cerevisiae* that has a genotype *pdcl(-6,-2)::loxP pdc5(-6,-2)::loxP pdc6(-6,-2)::loxP ura3-52 YEplLDH*.

Claim 12. (Original) The method of claim 1, wherein the culturing is performed in an aerobic batch culture, in an aerobic fed-batch culture, or in an aerobic chemostat.

Claim 13. (Previously presented) The method of claim 1, wherein the AT yeast strain is C<sub>2</sub> carbon source independent.

Claim 14. (Original) The method of claim 13, wherein the first culture medium is a minimal medium comprising at least one defined carbon source selected from the group consisting of glucose, sucrose, fructose, maltose, lactose, and galactose.

Claim 15. (Original) The method of claim 14, wherein glucose is the sole carbon source.

Claim 16. (Previously presented) The method of claim 1, wherein the AT yeast strain is C<sub>2</sub> carbon source dependent and the first culture medium is a minimal medium comprising a carbon source consisting essentially of glucose and at least one C<sub>2</sub> carbon source.

Claim 17. (Original) The method of claim 1, wherein the first culture medium consists essentially of at least one defined carbon source, at least one nitrogen source, monopotassium phosphate, magnesium sulfate, copper sulfate, ferric chloride, manganese sulfate, sodium molybdate, zinc sulphate, biotin, inositol, thiamine, and water, wherein the nitrogen source is selected from the group consisting of urea, ammonium sulfate, ammonium phosphate, and ammonium nitrate.

Claim 18. (Original) The method of claim 1, wherein a chromosome of the AT yeast strain comprises the exogenous lactate dehydrogenase gene.

Claim 19. (Original) The method of claim 1, wherein at least one plasmid comprising the exogenous lactate dehydrogenase gene is present in the AT yeast strain.

Claim 20. (Original) The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum*, bovine, *Lactobacillus casei*, *Bacillus megaterium*, *Rhizopus oryzae*, or *Bacillus stearothermophilus* lactate dehydrogenase gene.

Claim 21. (Original) The method of claim 1, wherein the exogenous lactate dehydrogenase gene is a *Lactobacillus plantarum* lactate dehydrogenase gene.

Claim 22. (Original) The method of claim 1, further comprising the step of recovering and purifying the lactic acid or a salt thereof.

Claim 23. (Original) The method of claim 22, wherein the purification step comprises at least one of distillation, ion exchange, nanofiltration or solvent extraction.

Claims 24-128. (Cancelled)

Claim 129. (Previously presented) The method of claim 1, wherein performing selection comprises:

- growing the parent yeast strain aerobically in a minimal medium, to yield a parent yeast strain culture;

- approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid;

- removing an aliquot of the parent yeast strain culture when the parent yeast strain culture is at about the lowest pH;

- seeding a minimal medium with the aliquot of the parent yeast strain culture; and

- repeating the growing, approximating, removing, and seeding steps until a final lowest pH reaches a value lower than the lowest pH of the first approximating and first removing steps, to yield an acid-tolerant yeast strain.

Claim 130. (Previously presented) The method of claim 1, wherein performing selection comprises:

- growing the parent yeast strain aerobically in a minimal medium, to yield a parent yeast strain culture;

- approximating the lowest pH of the parent yeast strain culture at which the parent yeast strain will grow and produce lactic acid;

- removing an aliquot of the parent yeast strain culture when the parent yeast strain culture ceases growth;

- seeding a minimal medium with the aliquot; and

repeating the approximating, removing, and seeding steps until a final lowest pH reaches a value lower than the lowest pH of the first approximating and first removing steps, to yield an acid-tolerant yeast strain.

## **EVIDENCE APPENDIX**

None.

## **RELATED PROCEEDINGS APPENDIX**

None.